JC02 Rec'd PCT/PTO 27 MAR 2002

FORM PTO-1390 (REV. 12-2001) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 930008-2069
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	U S APPLICATION NO (If known see 37 C F R 1 5) 10/089392
INTERNATIONAL APPLICATION NO. PCT/EP00/07115	INTERNATIONAL FILING DATE 25 July 2000 (25.07.00)
	PRIORITY DATE CLAIMED 30 September 1999 (30.09.99)
TITLE OF INVENTION	ESTER-GROUP-CLEAVING ENZYME FROM THERMOMONOSPORA FUSCA
APPLICANT(S) FOR DO/EO/US	**Wolf-Dieter Deckwer; Rolf-Joachim Mueller; Ilona Kleeberg; and Joop Van Den Heuvel**

Applicants herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.4. ☐ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U S.C. 371(c)(4)).
10. ☒ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 – 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4)
20. ☒ Other items or information:
    International Search Report; IPER with annexes; WIPO cover page

EXPRESS MAIL

Mailing Label Number. **EV001583515US**

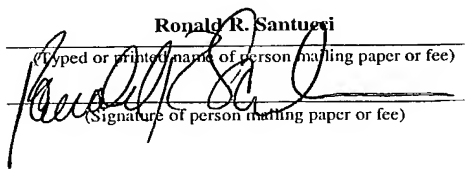
Date of Deposit **March 27, 2002**

I hereby certify that this paper or fee is being deposited with the United States Postal Service

"Express Mail Post Office to Addressee" Service under 37 CFR 1 10 on the date indicated above and is addressed to the Assistant Commissioner for Patents and Trademarks, Box PCT Washington, DC 20231.

Ronald R. Santucci

(Typed or printed name of person mailing paper or fee)



(Signature of person mailing paper or fee)

00057746

Express Mail No.EV001583515US

U.S. APPLICATION NO (If known, see 37 CFR 1.5-50)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NO
10/089392	PCT/EP00/07115	930008-2069

21. ☒ The following fees are submitted	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37CFR 1 445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... $1040.00 International preliminary examination fee (37 C F R 1 482) not paid to USPTO but International Search Report prepared by the EPO or JPO . . . .. $890 00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .. . . $740.00 International preliminary examination fee paid to USPTO (37 CFR 1 482) but all claims did not satisfy provisions of PCT Article 33(1)-(4 . . . . $710 00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100 00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$ 890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	16 - 20 =		x $18.00	$	
Independent Claims	2+ - 3 =		x $84.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	$	

TOTAL OF ABOVE CALCULATIONS =	$ 890.00	
☐ Applicant claims small entity status. See 37 C.F.R. 1.27. The fees indicated above are reduced by ½. +	$	
SUBTOTAL =	$	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	$	
TOTAL NATIONAL FEE =	$ 890.00	
Fee for recording the enclosed assignments (37 CFR 1 21(h)) The assignment must be accompanied by an appropriate cover sheet (37 CFR 3 28, 3.31) **$40.00** per property +	$	
TOTAL FEES ENCLOSED =	$ 890.00	
	Amount to be refunded:	$
	charged:	$

a. ☒ A check in the amount of $**890.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-0320**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. WARNING: Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Ronald R. Santucci
Frommer Lawrence & Haug LLP
745 Fifth Avenue
New York, New York 10151

SIGNATURE

Ronald R. Santucci

NAME

28,988

REGISTRATION NUMBER

00057746

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Wolf-Dieter Deckwer, Rolf-Joachim Mueller, Ilona Kleeberg, And Joop van den Heuvel
Serial No.	:	Not Yet Assigned
For	:	ESTER-GROUP-CLEAVING ENZYME FROM THERMOMONOSPORA FUSCA
Filed	:	
Int'l Appln. No.	:	PCT/EP00/07115
Int'l Filing Date	:	25 July 2000 (25.07.00)
Priority Date	:	30 September 1999 (30.09.99)
Examiner	:	Not Yet Assigned
Art Unit	:	Not Yet Assigned

745 Fifth Avenue
New York, NY 10151

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to the examination of the above referenced patent application,

Applicants respectfully request that the application be amended as follows:

00058464

In the Claims:

Please rewrite claims 3-5, 7-8, 10 and 13 as follows:

3. (Amended) Ester-group-cleaving enzyme according to claim 1, the enzyme being isolated from the nutrient medium by obtaining an enzyme-containing culture supernatant from the nutrient medium, which supernatant may optionally be concentrated, and purifying the enzyme by chromatography, especially by ion exchange chromatography and/or hydrophobic interaction chromatography.

4. (Amended) Ester-group-cleaving enzyme according to claim 1, the enzyme being characterised by the following parameters:

molecular weight: 27400 d (determined by SDS gel electrophoresis) or 28200 d (calculated on the basis of the amino acid sequence),

temperature optimum/range: 65°C (30-80°C),

temperature stability: 70°C/30 min,

pH optimum/range: 6-7 (4- >8),

isoelectric point: 6.4.

5. (Amended) Ester-group-cleaving enzyme according to claim 1, characterised by the following amino acid sequence:

ANPYERGPNP	TDALLEASSG	PFSVSEENVS	RLSASGFGGG
TIYYPREN	NTYGAVAISP	GYTGTEASIA	WLGERIASHG
FVVITIDTIT	TLDQPDSRAE	QLNAALNHMI	NRASSTVRSR
IDSSRLAVMG	HSMGGGGTLR	LASQRPDLKA	AIPLTPWHLN
KNWSSVTVPT	LIIGADLDTI	APVATHAKPF	YNSLPSSISK

AYLELDGATH FAPNIPNKII GKYSVAWLKR FVDNDTRYTQ
FLCPGPRDGL FGEVEEYRST CPF

or

mutations resulting from substitution, insertion or deletion of amino acids, which mutations cleave ester groups of polyesters (isofunctional enzymes).

7. (Amended) Polyclonal antibody directed specifically against an ester-cleaving enzyme according to claim 1 or against a synthetic peptide or protein.

8. (Amended) Monoclonal antibody directed specifically against an ester-cleaving enzyme according to claim 1 or against a synthetic peptide or protein.

10. (Amended) Ester-group-cleaving composition that comprises an ester-group-cleaving enzyme according to claim 1 and/or a synthetic peptide or protein and optionally additional enzymes, stabilisers, suitable surface-active substances and/or suitable organic solvents.

13. (Amended) Use of an ester-group-cleaving enzyme according to claim 1 or of a synthetic peptide or protein or of an ester-group-cleaving composition for the degradation of ester-group-containing low molecular weight and/or macromolecular synthetic or natural compounds.

REMARKS

The claims of the above referenced application (as they have been amended under Article 34 and are attached as annexes to the International Preliminary Examination Report) have been amended to remove all multiple dependencies. No new matter has been added. Accordingly, an early examination of the application is respectfully requested.

The Commissioner is authorized to charge any additional fees that may be required to Deposit Account No. 50-0320.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP

By: _____
Ronald R. Santucci
Reg. No. 28,988
(212) 588-0800

APPENDIX: (claims with markings)

3. (Amended) Ester-group-cleaving enzyme according to [either of the preceding claims] claim 1, the enzyme being isolated from the nutrient medium by obtaining an enzyme-containing culture supernatant from the nutrient medium, which supernatant may optionally be concentrated, and
purifying the enzyme by chromatography, especially by ion exchange chromatography and/or hydrophobic interaction chromatography.

4. (Amended) Ester-group-cleaving enzyme according to [any one of the preceding claims] claim 1, the enzyme being characterised by the following parameters:

molecular weight: 27400 d (determined by SDS gel electrophoresis) or 28200 d (calculated on the basis of the amino acid sequence),

temperature optimum/range: 65°C (30-80°C),

temperature stability: 70°C/30 min,

pH optimum/range: 6-7 (4- >8),

isoelectric point: 6.4.

5. (Amended) Ester-group-cleaving enzyme according to [any one of the preceding claims] claim 1, characterised by the following amino acid sequence:

ANPYERGPNP	TDALLEASSG	PFSVSEENVS	RLSASGFGGG
TIYYPREN	NTYGAVAISP	GYTGTEASIA	WLGERIASHG
FVVITIDTIT	TLDQPDSRAE	QLNAALNHMI	NRASSTVRSR
IDSSRLAVMG	HSMGGGGTLR	LASQRPDLKA	AIPLTPWHLN
KNWSSVTVPT	LIIGADLDTI	APVATHAKPF	YNSLPSSISK

AYLELDGATH FAPNIPNKII GKYSVAWLKR FVDNDTRYTQ
FLCPGPRDGL FGEVEEYRST CPF

or

mutations resulting from substitution, insertion or deletion of amino acids, which mutations cleave ester groups of polyesters (isofunctional enzymes).

7. (Amended) Polyclonal antibody directed specifically against an ester-cleaving enzyme according to [any one of claims 1 to 5] claim 1 or against a synthetic peptide or protein [according to claim 6].

8. (Amended) Monoclonal antibody directed specifically against an ester-cleaving enzyme according to [any one of claims 1 to 5] claim 1 or against a synthetic peptide or protein [according to claim 6].

10. (Amended) Ester-group-cleaving composition that comprises an ester-group-cleaving enzyme according to [any one of claims 1 to 5] claim 1 and/or a synthetic peptide or protein [according to claim 6] and optionally additional enzymes, stabilisers, suitable surface-active substances and/or suitable organic solvents.

13. (Amended) Use of an ester-group-cleaving enzyme according to [any one of claims 1 to 5] claim 1 or of a synthetic peptide or protein [according to claim 6] or of an ester-group-cleaving composition [according to any one of claims 10 to 12] for the degradation of ester-group-containing low molecular weight and/or macromolecular synthetic or natural compounds.

Gesellschaft für Biotechnologische
Forschung GmbH

Our reference: 10892
New International Patent Application

Ester-group-cleaving enzyme from Thermomonospora fusca

The invention relates to an ester-group-cleaving enzyme (hereinafter also referred to as EGC enzyme) from Thermomonospora fusca, to a method for the preparation thereof and to its use in the degradation and treatment of ester-group-containing polymers and low molecular weight compounds.

Introduction and state of the art

Polymers and macromolecular materials that are susceptible to controlled biological degradation have become increasingly important in recent years. A number of such products are already commercially available on an industrial scale. Of those novel products, ester-group-containing polymers (e.g. polyesters, polyesterurethanes, polyesteramides) play a central role. Examples of biodegradable polyester-based plastics are, for example, poly(β-hydroxybutyrate-co-β-hydroxyvalerate), poly(ε-caprolactone) and poly(butylene succinate).

Since polymers cannot, owing to their molecular size, penetrate the outer membrane of microbial cells, the first step in degradation, which generally determines the rate, is a reduction in molecular weight (depolymerisation) by extracellular enzymes. Polyesters are accordingly potentially biodegradable, since the ester bonds constitute basic points of attack for such extracellular hydrolysing enzymes.

In the case of aliphatic polyesters, studies into biological degradation using such hydrolysing enzymes (e.g. lipases, PHB depolymerases) have been known for a long time [Tokiwa *et al.*, Polym. Mater. Sci. Eng. 62 (1990), 988-992] [Jendrossek *et al.*, Appl. Microbiol. Biotechnol. 46 (1996), 451-4631]. The material is incubated under suitable conditions with a corresponding enzyme and the degradation is determined by the formation of cleavage products in the surrounding medium or by the weight loss of the samples. In the case of natural polyhydroxyalkanoates, there were generally used hydrolases (PHB depolymerases) that had been specially isolated for the purpose, whereas for the degradation of synthetic polyesters there were used commercial lipases etc. that had not been specially isolated for the purpose of polymer degradation.

Whilst many aliphatic polyesters have proved in principle to be susceptible to biological attack, aromatic polyesters [e.g. poly(ethylene terephthalate), poly(propylene terephthalate), polybutylene terephthalate)] are known to be biologically resistant. In order to utilise the processing and application properties of the aromatic structures, which are better than those of aliphatic polyesters, in recent years biodegradable aliphatic-aromatic copolyesters have been developed and are manufactured on an industrial scale [Press information from BASF AG, Ludwigshafen, for the K' 98 Trade Fair in Düsseldorf, of 17.03.98].

As a result of the introduction of aromatic components, however, the rate of biological degradation is significantly reduced [Müller *et al.*, Polym. Degrad. Stab. 59 (1998), p. 203-208]. Thus, for example, Jun *et al.*, [Jun *et al.*, J. Environ., Polym. Degrad. 2(1) (1994), p. 9-18] come to the conclusion that copolyesters of PET and PCL are not significantly attacked by lipases (e.g. Pseudomonas sp. lipase).

Degradation of, in particular, polyesteramides using various customary commercial lipases has recently been described in terms of technical aspects [WO 98/36086]. That Patent Specification also describes the decomposition of a copolyester of butanediol, terephthalate (40 mol %) and adipate (60 mol %). The reactions, which are claimed to be

suitable for technical applications, are achieved by, for example, 50 mg of enzyme (lipase from Candida antarctica) to 0.3-1.8 g of a polyesteramide in film or plate form. The rates of degradation obtained are in the region of 600 mg degradation/week. For the described degradation of the aliphatic-aromatic copolyester, an amount of enzyme of 1% (in 100 ml of buffer) must be added to a fine powder of the copolyester. Despite the considerably larger surface area covered as a result of the small particle size, a degradation of only 230 mg/week is obtained.

It has recently been shown that aliphatic-aromatic copolyesters can be degraded by microorganism strains from the group of Actinomycetes [Kleeberg *et al.*, Appl. Environ. Polym. Degrad. 64(5), (1998), 1731-1735].

Nonetheless, there is still a need for a highly active ester-group-cleaving enzyme that is capable of degrading polyester-based polymers.

Surprisingly, it has been found, according to the invention, that biodegradable polyester-group-containing polymers, especially also aliphatic-aromatic copolyesters, can be depolymerised and broken down into low molecular weight fragments at an exceptionally high rate of degradation using the extracellular enzyme according to the invention, specified in more detail hereinafter, from the microorganism Thermomonospora fusca, which belongs to the Actinomycetes, especially the strain Thermomonospora fusca DSM 43793, on its own or in a mixture with other enzymes.

The invention accordingly relates to an ester-group-cleaving enzyme according to patent claim 1, a synthetic peptide or protein according to patent claim 6, polyclonal and monoclonal antibodies according to patent claims 7 and 8, hybridoma cells according to patent claim 9, an ester-group-cleaving composition according to patent claim 11 and to the use of an ester-group-cleaving enzyme, synthetic peptide or protein or of an ester-group-cleaving composition according to patent claim 13.

Advantageous embodiments are given in the dependent claims.

More specifically, but without implying any limitation, the invention relates to an ester-group-cleaving enzyme obtainable by culturing the microorganism Thermomonospora fusca in a suitable nutrient medium, optionally in the presence of an inducer.

Preferably, the ester-group-cleaving enzyme according to the invention originates from the Thermomonospora fusca strain that has been deposited with the Deutschen Sammlung für Microorganismen [German Collection of Microorganisms] under the number DSM 43793.

The culture can be carried out by batch, fed batch or continuous operation in synthetic or complex media. The microorganisms can be free or immobilised on a solid carrier. In principle, both natural and genetically modified microorganisms are suitable.

Suitable inducers for the production of the enzyme are, for example, the substrates themselves, e.g. aliphatic polyesters and/or oligoesters, aliphatic-aromatic copolyesters.

In a preferred embodiment, the ester-group-cleaving enzyme according to the invention is also isolated from the nutrient medium by obtaining an enzyme-containing culture supernatant from the nutrient medium, for example by centrifugation, which supernatant may optionally be concentrated, for example by ultrafiltration and/or ammonium sulphate precipitation, whereupon the enzyme is purified by customary biochemical methods of purification, for example by chromatography, especially by ion exchange chromatography and/or hydrophobic interaction chromatography.

The ester-group-cleaving enzyme from Thermomonospora fusca DSM 43793 according to the invention is characterised by the following parameters:

molecular weight: 27400 d (determined by SDS gel electrophoresis) or 28200 d (calculated on the basis of the amino acid sequence);

temperature optimum/ range: 65°C (30-80°C);

temperature stability: 70°C/30 min.;

pH optimum/range: 6-7 (4- >8);

isoelectric point: 6.4.

The substrate specificity encompasses ester-group-containing polymers, triglycerides and phthalic acid esters.

According to a preferred embodiment, the ester-group-cleaving enzyme from Thermomonospora fusca DSM 43793 according to the invention has the following amino acid sequence:

ANPYERGPNP	TDALLEASSG	PFSVSEENVS	RLSASGFGGG
TIYYPREN	NTYGAVAISP	GYTGTEASIA	WLGERIASHG
FVVITIDTIT	TLDQPDSRAE	QLNAALNHMI	NRASSTVRSR
IDSSRLAVMG	HSMGGGGTLR	LASQRPDLKA	AIPLTPWHLN
KNWSSVTVPT	LIIGADLDTI	APVATHAKPF	YNSLPSSISK
AYLELDGATH	FAPNIPNKII	GKYSVAWLKR	FVDNDTRYTQ
FLCPGPRDGL	FGEVEEYRST	CPF	

or

a mutated amino acid sequence resulting from substitution, insertion or deletion of amino acids, which mutated amino acid sequence yields an isofunctional enzyme.

The above amino acid sequence or parts thereof can of course also be prepared synthetically by conventional methods, for example using an automatic peptide synthesizer.

The invention relates also to polyclonal and monoclonal antibodies that are directed specifically against an ester-cleaving enzyme according to the invention or against a corresponding synthetic peptide or protein having identical function and/or amino acid sequence, and also to hybridoma cells that produce the monoclonal antibodies. The preparation of poly-or mono-clonal antibodies and the preparation of hybridomas that produce the latter have been known for a long time (see, for example: E. Harlow, D. Lane, "Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory, 1988; E. Lidell, I. Weeks, "Antikörper-Techniken", Spektrum Akademischer Verlag, 1996), and so require no further explanation.

The invention relates also to ester-group-cleaving compositions that comprise an ester-group-cleaving enzyme according to the invention and/or a corresponding synthetic peptide or protein having identical function and/or amino acid sequence and optionally additional enzymes, stabilisers, suitable surface-active substances and/or suitable organic solvents.

Preferably the additional enzymes are hydrolases, especially esterases, proteases, cutinases, lipases, phospholipases and lysophospholipases.

Especially preferably those hydrolases originate from microorganisms selected from Pseudomonas sp., Rizomucor miehei, Candida cylindracea, Candida antartica, Aspergillus niger, Chromobacterium viscosum, Commamonas acidovorans, Rhizopus arrhizus and Rhizopus delamar. Especially suitable are also the microroganisms disclosed in WO 98/36086 (Bayer AG), to which reference is expressly made herein.

The invention relates also to the use of an ester-group-cleaving enzyme according to the invention or of a synthetic peptide or protein having identical function and/or amino acid sequence or of an ester-group-cleaving composition according to the invention for the degradation of ester-group-containing low molecular weight and/or macromolecular synthetic or natural compounds.

Preferably the ester-group-containing macromolecular compounds are aliphatic, cycloaliphatic, aliphatic-aromatic, partially aromatic or aromatic polyesters or copolyesters, polyesteramides, polyestercarbonates or polyesterurethanes, the chain of which may be extended and which may be branched or crosslinked.

The ester-group-containing macromolecular compounds can be in any desired form and can form, for example, copolymers, mixtures and blends, composites, laminates or adhesive bonds with other materials.

In the method for the degradation of ester-group-containing low molecular weight and/or macromolecular (polymeric) compounds using the ester-group-cleaving enzyme according to the invention (or an enzyme prepared synthetically from the amino acid sequence) or a composition comprising such an enzyme, it is possible to achieve rates of decomposition that are markedly superior to those of systems known hitherto and that enable technical exploitation of the enzymatic treatment of ester-group-containing polymers. This is especially the case for aliphatic-aromatic copolyesters and polyester blends, which are economically very important.

The use of the ester-group-cleaving enzyme according to the invention (or of an enzyme prepared synthetically from the amino acid sequence) or of a composition comprising such enzymes in the treatment of the polymers mentioned above and hereinbelow in technically relevant forms, for example films, injection-moulded parts, coatings, laminates, foams, particles, adhesive bonds, can be used to increase the rate of metabolisation by microorganisms, to process products in the context of recycling (e.g. to dissolve adhesive bonds or remove coatings), to recover polymer building blocks from biodegradable polymers or to modify the surface of products made from polyesters.

The treatment of the polymers with a suitable enzyme formulation, for example in the form of a crude culture supernatant of Thermomonospora fusca, which may optionally be concentrated, a purified enzyme or a synthetic enzyme or a composition comprising such

enzymes, can be effected, for example, in an aqueous solution or by application of the enzyme formulation to the polymeric materials.

Low molecular weight ester compounds play a part as additives in various polymers. Such compounds can also be cleaved by the enzyme according to the invention.

The ester-group-containing polymers that can be degraded by the enzyme according to the invention (or by an enzyme prepared synthetically from the amino acid sequence) and/or by the composition comprising such enzymes include, in addition to the polymers already mentioned above, for example, the following:

ester-group-containing synthetic and natural polymers, especially lignins, lignocellulose, cutin, suberin, aliphatic polyesters, especially those disclosed in WO 98/36086 (Bayer AG), to which reference is expressly made herein, especially polycaprolactone, aromatic or partially aromatic copolyesters, especially those disclosed in WO 98/36086 (Bayer AG), especially those containing terephthalic acid, more especially copolyesters of 1,4-butanediol, terephthalic acid and adipic acid (BTA), especially containing 30-70 mol % of terephthalic acid, polyesteramides, especially those disclosed in WO 98/36086 (Bayer AG), polymers containing urethane and ester groups, that is to say polyesterurethanes, and segmented polyurethanes.

The chain of the polyesters may be extended and the polyesters may be branched or crosslinked.

Especially preferred specific polyesters are poly(propylene succinate), poly(butylene succinate), poly(butylene succinate-co-ethylene succinate), a copolymer of succinic acid/adipic acid/1,2-ethanediol/1,4-butanediol, copolymers of 1,4-butanediol/adipic acid/terephthalic acid.

The ester-group-containing polymers that can be degraded by the enzyme according to the invention (or by an enzyme prepared synthetically from the amino acid sequence)

and/or by the composition comprising such enzymes may be present, for example, in the form of:

copolymers or mixtures (blends) of two or more of the above-mentioned polymers;

composites or laminates of two or more of the above-mentioned polymers or copolymers or blends thereof;

composites, laminates or adhesive bonds with natural or modified natural polymeric materials, especially starch and/or cellulose (e.g. paper);

composites, laminates or adhesive bonds with other materials that are not necessarily biodegradable (e.g. glass);

polymer formulations comprising customary fillers, fibre reinforcers, auxiliaries, stabilisers.

The use according to the invention includes the treatment of polymers in the form of particles, suspensions, emulsions, coatings, adhesive bonds, films, mouldings, fibres or webs, wovens and foams. The materials can be used untreated or pretreated chemically, thermally or mechanically.

The enzyme is used, for example, in a buffered solution or in an unbuffered solution, optionally with adjustment of the pH value.

The application is effected, for example, by introducing ester-group-containing substances into suitable enzyme solutions or by applying a suitable enzyme formulation to corresponding substance surfaces.

Further possible uses of the enzyme according to the invention relate to the treatment of the above-defined materials for the purpose of pre-treatment in the course of disposal, the

treatment of materials for the purpose of separating product components, the treatment of materials for the purpose of recovering individual material constituents or all the material constituents and the treatment of materials for the purpose of altering surface properties.

The following Examples serve to illustrate the invention and are not to be regarded as limiting.

1. Culture of Thermomonospora fusca DSM 43793.

A sterile culture flask without baffles, which can be sealed with an aluminium lid, is filled to two centimetres with sterile medium (corresponding to DIN V 54900, Part 2). 3 g/litre of a copolyester synthesised from 1,4-butanediol, terephthalic acid ester and adipic acid are added to the flask and inoculated with 1 % by volume of the inoculum from a preculture of Thermomonospora fusca. The culture is incubated for 18 hours at 55°C on a rotary shaker at 120 rev/min.

After stopping the culture, the solids are removed by centrifugation at 8000 x g for 20 minutes at 10°C. The supernatant contains the ester-cleaving enzyme.

2. Degradation of an aliphatic-aromatic copolyester with Thermomonospora fusca in the culture supernatant.

Thermomonospora fusca DSM 43793 is cultured in a mineral salt medium (see Example 1) for 24.8 hours at 55°C. 2 ml of the organism-free culture supernatant are introduced into a test tube. A circular polymer film (diameter 0.9 cm) of a copolyester of butanediol, terephthalic acid and adipic acid (40 mol % terephthalic acid in the acid component) is added to the culture supernatant and incubated for 24 hours at 55°C, after which the weight loss of the film is 2.575 mg/cm^2 surface area.

3. Isolation of the ester-cleaving enzyme according to the invention.

Concentration:

The culture supernatant of Example 1 is concentrated to 5% of the original volume in an Amicon ultrafiltration chamber (volume: 50 ml, filtration surface area: 47 mm^2) at a pressure of 3 bar and using a membrane having a cut-off of 10 Kd.

Further purification is effected using a standard FPLC system, "LCC-Plus", having automatic equilibration, injection and elution (Pharmacia, Uppsala, Sweden). The concentrated protein in the culture supernatant (2.1 mg) is purified in a first step over an ion exchange column.

Parameters:

Column: UNO-S1 column (column volume 1.3 ml, BioRad, Munich)

Starting buffer: 20 mM citrate buffer (pH 4.0)

Elution: (linear gradient) 1M NaCl in the starting buffer

Flow rate: 2 ml/min

Figure 1 shows the elution profile, the black band indicating the fractions that exhibit ester-group-cleaving activity.

In a second step, fractions that are obtained by ion exchange chromatography and that exhibit activity are purified further by hydrophobic interaction chromatography (HIC).

116 µg of protein from fractions obtained by ion exchange chromatography are applied to a Phenylsepharose column.

Column: Phenylsepharose-CL4B column (column volume: 1.14 ml, Pharmacia. Uppsala, Sweden)

Starting buffer: 0.5M ammonium sulphate in 20 mM phosphate buffer (pH 7.1)

Elution: (stepped gradient) 30 % isopropanol in 20 mM phosphate buffer (pH 7.1)

Flow rate: 0.3 ml/min.

Figure 2 shows the elution profile, the black band indicating the fractions that exhibit ester-group-cleaving activity.

The culture supernatant has a specific activity of 3.3 U/mg. After ion exchange chromatography a specific activity of 218 U/mg is obtained and after HIC a specific activity of 360 U/mg is obtained.

Characterisation of the enzyme according to the invention.

Figure 3 shows the amino acid sequence of the enzyme according to the invention and the alignment, for the purpose of sequence comparison, with triacylglycerol-lipase from Streptomyces albus G and with triacylglycerol-acylhydrolase from Streptomyces sp. M11. The multiple alignment was produced using the "PileUp" program (Wisconsin Package, Version 9.1, Genetics Computer Group, Madison, WI, USA). Amino acids differing from one another at identical positions are shown shaded. The black-rimmed box indicates a highly conserved amino acid sequence from the region of the active centre of lipases. The sequences of the two Streptomyces strains originate from the SP-TREMBL Databank (Release 7.0, 08/1998): Q56008 (Streptomyces sp. M11), Q59798 (Streptomyces albus G).

For the amino acid sequencing, the EGC enzyme was isolated from the foreign proteins still present after purification. This was carried out by separating out the proteins by

means of preparative SDS gel electrophoresis and transfer to a PVDF membrane by Western blotting. After staining of the protein bands, the enzyme band was cut out from the membrane and sequenced.

In order to determine the entire sequence, the enzyme was digested with trypsin and GIuC. The separation of the resulting peptides was effected by HPLC (reversed phase). The N-terminal sequence and the peptide fractions from the digestion of the BTA-hydrolase were analysed by Edman degradation in an Applied Biosystems 473A Sequencer (gas-phase mode) or in a 494A Procise HT Sequencer (gas-phase and pulsed-liquid mode) using standard programs from the manufacturer.

The entire sequence of the enzyme was determined by sequence overlapping and comparison of the partial sequences of the EGC enzyme with the amino acid sequences of two known Streptomyces lipases.

4. Degradation of ester-group-containing polymers using the ester-group-cleaving enzyme according to the invention

Under sterile conditions in test tubes, 1 ml of the purified enzyme solution (25 µg of enzyme in 20 mM phosphate buffer, pH 7.1) was added to a polymer film (d = 0.9 cm). The test tubes were incubated for 17 hours at 55°C. The weight loss of the polymer films served as a measure of the enzyme activity.

In addition to the aliphatic-aromatic copolyesters BTA40:60 (40 mol % terephthalic acid in the acid component) and BTA60:40 (60 mol% terephthalic acid in the acid component), the following are subjected to degradation: an aliphatic polyester SP3:13 (synthesised from 1,3-propanediol and brassylic acid) and the commercial ester-group-containing polymers Bayer Tir 1874 (polyesteramide from Bayer AG), Bionolle (aliphatic polyester from Showa Highpolymers) and the natural bacterial polyester P(3HB). In respect of P(3HB), the ester-group-cleaving enzyme has no discernible activity. Bayer Tir 1874 was already fully solubilised at the time the sample was taken

and the activity indicated represents a minimum value. The results are shown in Figure 4.

5. Comparison of the ester-cleaving enzyme according to the invention with the lipase of Pseudomonas sp.

BTA40:60 films are each introduced into 6 ml of physiological sodium chloride solution (pH 7.0). 50 µg of the enzyme in question (ester-cleaving enzyme according to the invention or lipase of Pseudomonas sp. from SIGMA Chemical Co., EC 3.1.1.3) are added to the solution. The batch is incubated at the optimum temperature of the enzyme in question. The progress of degradation is monitored by titration of the free acids formed using 0.1M NaOH. The result is shown in Figure 5.

In comparison with Pseudomonas sp. lipase, it is possible to obtain a substantially higher rate of hydrolysis using the enzyme according to the invention.

6. Cleavage of triglycerides using the ester-cleaving enzyme according to the invention and using the lipase of Pseudomonas sp.

5 ml of an emulsion solution (4.475 g of NaCl, 0.103 g of KH_2PO_4 dissolved in a mixture of 75 ml of distilled water and 135 ml of glycerol (99.5%), to which 1.5 g of gum arabic is added, the solution being made up to 250 ml with distilled water) and 4.5 ml of distilled water are added to 0.5 ml of each of the triglycerides.

The substrate solution is made up directly before the start of the enzyme test and is homogenised using an Ultraturrax for 1 minute at 13500 rev/min.

The enzyme solution is then added to the substrate solution (20 µg of enzyme per 6 ml of substrate solution), the pH value is adjusted to pH 7.1 and the ester cleavage is monitored by titration using 0.1M NaOH. Figure 6 shows the results for triglycerides having different numbers of carbon atoms in the fatty acid component.

A broad spectrum of fatty acids can be cleaved.

7. Cleavage of phthalic acid esters using the ester-cleaving enzyme according to the invention and using the lipase of Pseudomonas sp.

The test batches correspond to those of Example 6. Phthalic acid esters having different alcohol components are used instead of the triglycerides. Whilst the lipase from Pseudomonas sp. is able to cleave only the dimethyl and diethyl esters, the enzyme according to the invention also hydrolyses esters having longer-chained alcohols. The rates of hydrolysis are higher than those of Pseudomonas sp. lipase. The results are shown in Figure 7.

Patent claims

1. Enzyme that cleaves ester groups of polyesters, which enzyme is obtainable by culturing the microorganism Thermomonospora fusca in a suitable nutrient medium in the presence of a polyester as inducer, obtaining from the nutrient medium a supernatant that contains an enzyme that cleaves ester groups of polyesters, purifying the enzyme by customary biochemical methods of purification and then isolating it.

2. Ester-group-cleaving enzyme according to claim 1, the microorganism being a Thermomonospora fusca strain that has been deposited with the Deutschen Sammlung für Mikroorganismen [German Collection of Microroganisms] under the number DSM 43793.

3. Ester-group-cleaving enzyme according to either of the preceding claims, the enzyme being isolated from the nutrient medium by obtaining an enzyme-containing culture supernatant from the nutrient medium, which supernatant may optionally be concentrated, and
purifying the enzyme by chromatography, especially by ion exchange chromatography and/or hydrophobic interaction chromatography.

4. Ester-group-cleaving enzyme according to any one of the preceding claims, the enzyme being characterised by the following parameters:

molecular weight: 27400 d (determined by SDS gel electrophoresis) or 28200 d (calculated on the basis of the amino acid sequence),

temperature optimum/range: 65°C (30-80°C),

temperature stability: 70°C/30 min,

pH optimum/range: 6-7 (4- >8),

AMENDED PAGE

isoelectric point: 6.4.

5. Ester-group-cleaving enzyme according to any one of the preceding claims, characterised by the following amino acid sequence:

ANPYERGPNP	TDALLEASSG	PFSVSEENVS	RLSASGFGGG
TIYYPREN	NTYGAVAISP	GYTGTEASIA	WLGERIASHG
FVVITIDTIT	TLDQPDSRAE	QLNAALNHMI	NRASSTVRSR
IDSSRLAVMG	HSMGGGGTLR	LASQRPDLKA	AIPLTPWHLN
KNWSSVTVPT	LIIGADLDTI	APVATHAKPF	YNSLPSSISK
AYLELDGATH	FAPNIPNKII	GKYSVAWLKR	FVDNDTRYTQ
FLCPGPRDGL	FGEVEEYRST	CPF	

or

mutations resulting from substitution, insertion or deletion of amino acids, which mutations cleave ester groups of polyesters (isofunctional enzymes).

6. Synthetic peptide or protein having the amino acid sequence of the ester-group-cleaving enzyme according to claim 5 or a part of the sequence thereof.

7. Polyclonal antibody directed specifically against an ester-cleaving enzyme according to any one of claims 1 to 5 or against a synthetic peptide or protein according to claim 6.

8. Monoclonal antibody directed specifically against an ester-cleaving enzyme according to any one of claims 1 to 5 or against a synthetic peptide or protein according to claim 6.

9. Hybridoma cell that produces a monoclonal antibody according to claim 8.

AMENDED PAGE

10. Ester-group-cleaving composition that comprises an ester-group-cleaving enzyme according to any one of claims 1 to 5 and/or a synthetic peptide or protein according to claim 6 and optionally additional enzymes, stabilisers, suitable surface-active substances and/or suitable organic solvents.

11. Ester-group-cleaving composition according to claim 10, wherein the additional enzymes are hydrolases, especially esterases, proteases, cutinases, lipases, phospholipases and lysophospholipases.

12. Ester-group-cleaving composition according to claim 11, wherein the hydrolases originate from microorganisms selected from Pseudomonas sp., Rizomucor miehei, Candida cylindracea, Candida antartica, Aspergillus niger, Chromobacterium viscosum, Commamonas acidovorans, Rhizopus arrhizus and Rhizopus delamar.

13. Use of an ester-group-cleaving enzyme according to any one of claims 1 to 5 or of a synthetic peptide or protein according to claim 6 or of an ester-group-cleaving composition according to any one of claims 10 to 12 for the degradation of ester-group-containing low molecular weight and/or macromolecular synthetic or natural compounds.

14. Use according to claim 13, wherein the ester-group-containing macromolecular compounds are aliphatic, cycloaliphatic, aliphatic-aromatic, partially aromatic or aromatic polyesters or copolyesters, polyesteramides, polyestercarbonates or polyesterurethanes, the chain of which may be extended and which may be branched or crosslinked.

15. Use according to claim 14, wherein the ester-group-containing macromolecular compounds form copolymers, mixtures and blends, composites, laminates or adhesive bonds with other materials.

AMENDED PAGE

16. Method for the preparation of an enzyme that cleaves ester groups of polyesters, wherein the microorganism Thermomonospora fusca is cultured in a suitable nutrient medium in the presence of a polyester as inducer, a supernatant containing an enzyme that cleaves ester groups of polyesters is obtained from the nutrient medium, and the enzyme is purified by customary biochemical methods of purification and then isolated.

AMENDED PAGE

Abstract

The invention relates to an ester-group-cleaving enzyme obtainable by culturing the microorganism Thermomonospora fusca in a suitable nutrient medium, optionally in the presence of an inducer.

Fig. 1

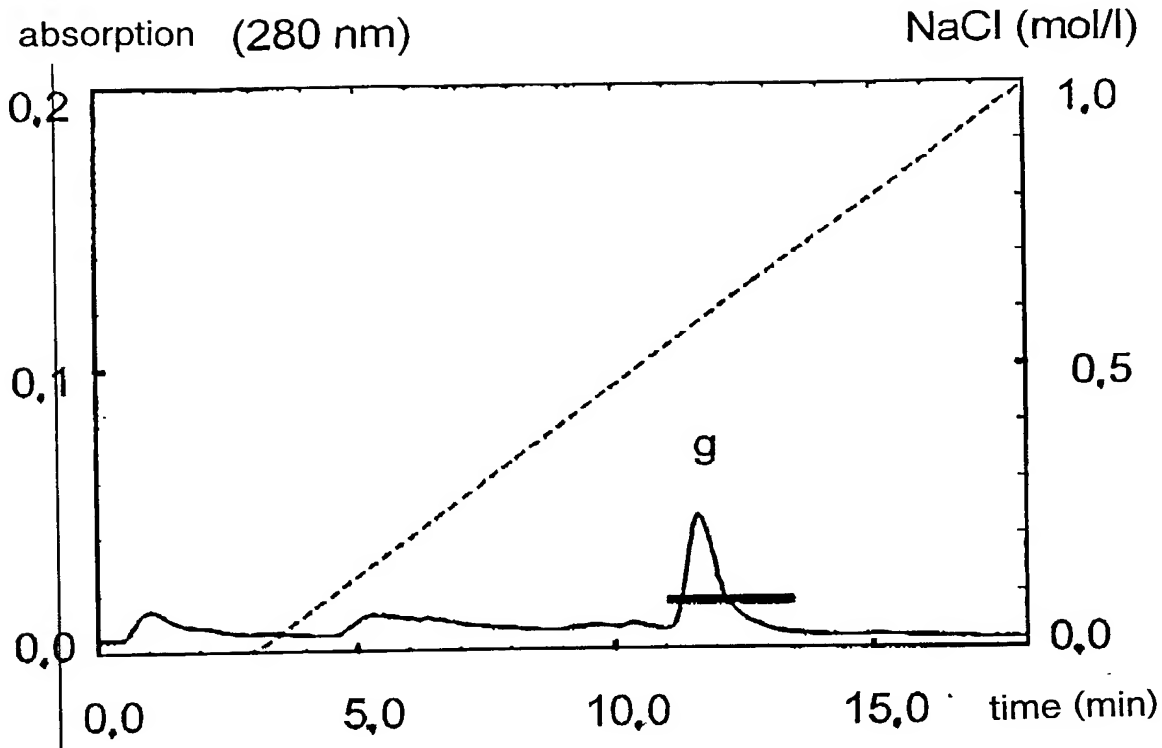

absorption (280 nm)
NaCl (mol/l)
0,2
0,1
0,0
1,0
0,5
0,0
g
0,0
5,0
10,0
15,0
time (min)

Fig. 2

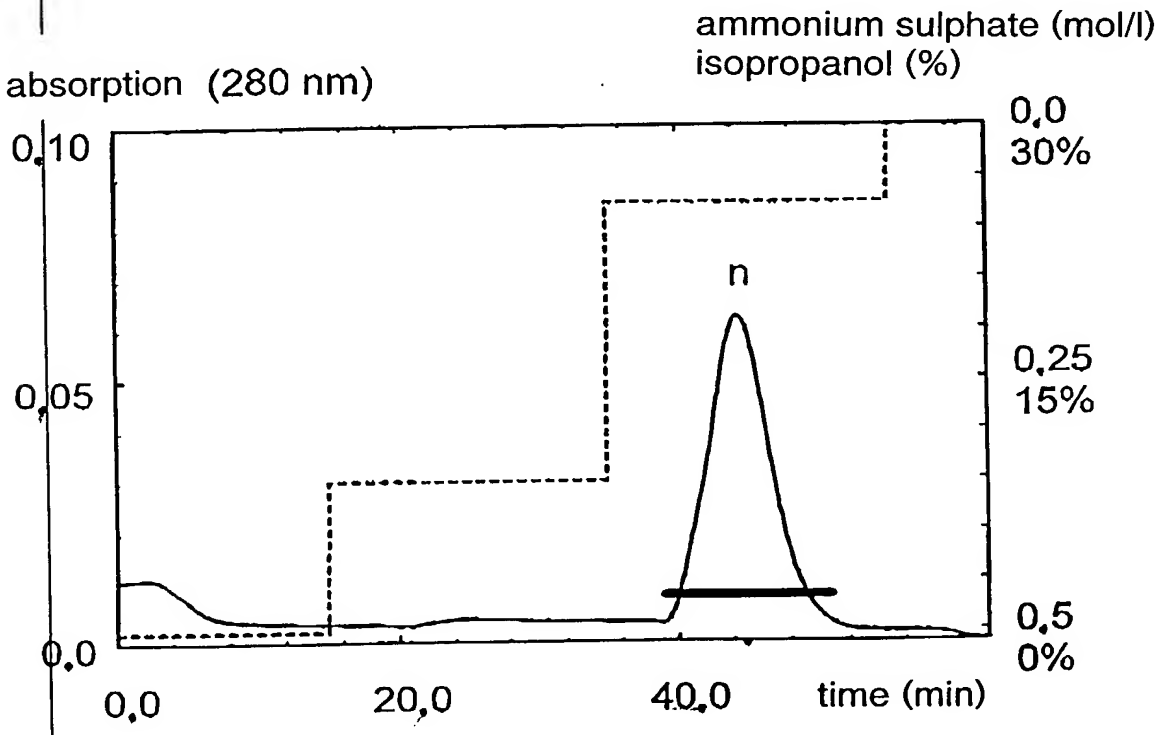

ammonium sulphate (mol/l)
isopropanol (%)
absorption (280 nm)
0,10
0,05
0,0
0,0
30%
0,25
15%
0,5
0%
n
0,0
20,0
40,0
time (min)

Fig. 3

```
Q59798        DNPYERGPA PTRASIEAPR GPYAVSQTSV SSLVVSGFGG      40
Q56008        ANPYERGPA PTNASIEASR GPYATSQTSV SSLVASGFGG      40
EGS-Enzym    .ANPYERGPN PTDALLEASS GPFSVSEENV SRLSASGFGG      39

Consensus    .ANPYERGPA PT.ASIEASR GPYAVSQTSV SSLVASGFGG      40


Q59798       GTIYYPTSTG DGTFGAVVVT PGFTATESSM AWLGPRLASQ      80
Q56008       GTIYYPTSTA DGTFGAVVIS PGFTAYQSSI AWLGPRLASQ      80
EGS-Enzym    GTIYYPRE-- NNTYGAVAIS PGYTGTEASI AWLGERIASH      77

Consensus    GTIYYPTST. DGTFGAVVIS PGFTATESSI AWLGPRLASQ      80


Q59798       GFVVFTIDTL TTLDQPDSRG RQMLAALDYL TER--SSART      118
Q56008       GFVVFTIDTN TTLDQPDSRG RQLLSALDYL TQR--SSVRT      118
EGS-Enzym    GFVVITIDTI TTLDQPDSRA EQLNAALNHM INRASSTVRS      117

Consensus    GFVVFTIDT. TTLDQPDSRG RQLLAALDYL T.R..SSVRT      120


Q59798       RIDGTRLGVI GHSMGGGGTL EAAKSRPSLK AAIPLTPWNL      158
Q56008       RVDATRLGVM GHSMGGGGSL EAAKSRTSLK AAIPLTGWNT      158
EGS-Enzym    RIDSSRLAVM GHSMGGGGTL RLASQRPDLK AAIPLTPWHL      157

Consensus    RID.TRLGVM GHSMGGGGTL E.AKSRPSLK AAIPLTPWNL      160


Q59798       DKTWPEVTTP TLVVGADGDT VAPVATHAKP FYSSLPSSTD      198
Q56008       DKTWPELRTP TLVVGADGDT VAPVATHSKP FYESLPGSLD      198
EGS-Enzym    NKNWSSVTVP TLIIGADLDT IAPVATHAKP FYNSLPSSIS      197

Consensus    DKTWPEVTTP TLVVGADGDT VAPVATHAKP FY.SLPSS.D      200


Q59798       RAYLELNNAT HFAPNLSNTT IAKYSVSWLK RFIDDDTRYE      238
Q56008       KAYLELRGAS HFTPNTSDTT IAKYSISWLK RFIDSDTRYE      238
EGS-Enzym    RAYLELDGAT HFAPNIPNKI IGKYSVAWLK RFVDNDTRYT      237

Consensus    RAYLEL.GAT HFAPN.SNTT IAKYSVSWLK RFID.DTRYE      240


Q59798       QFLCPLPVPD R--DIEEYRG TCPLGG      262
Q56008       QFLCPIPRPS L--TIAEYRG TCPHTS      262
EGS-Enzym    QFLCPGPRDG LFGEVEEYRS TCPF--      261

Consensus    QFLCP.PRP. L...IEEYRG TCP...      266
```

Q56008: triacylglycerol acyl hydrolase
Q59798: triacylglycerol lipase

Fig. 4

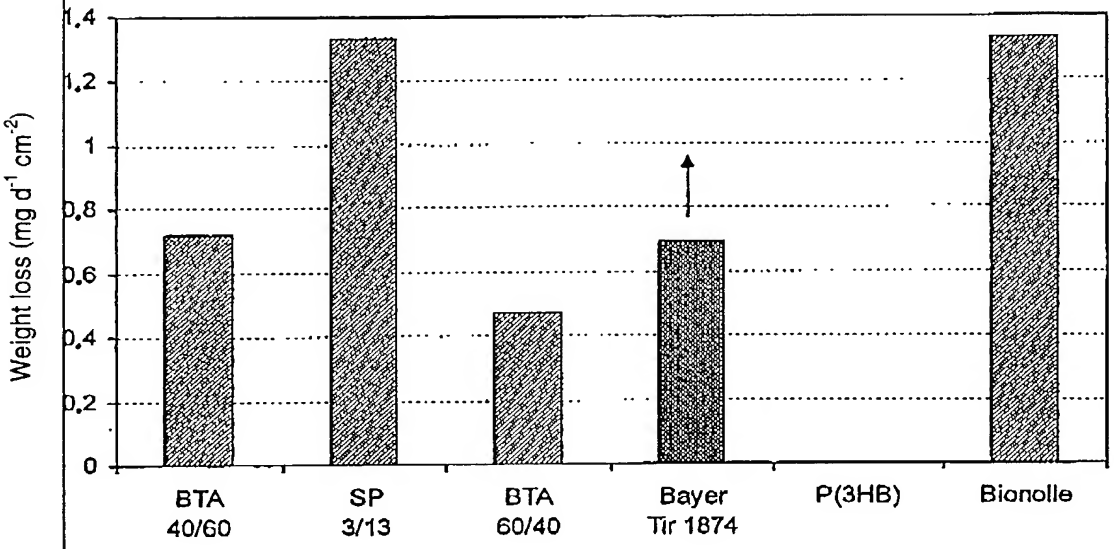

Weight loss (mg d-1 cm-2)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
BTA 40/60
SP 3/13
BTA 60/40
Bayer Tir 1874
P(3HB)
Bionolle

Fig. 5

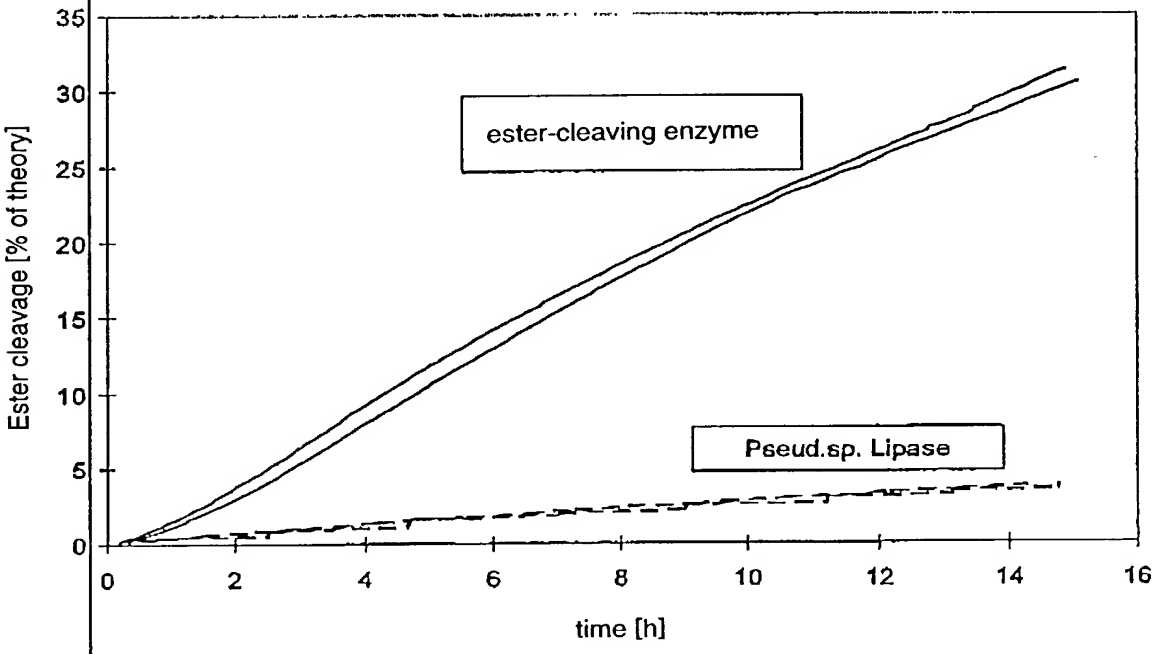

Ester cleavage [% of theory]
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
time [h]
ester-cleaving enzyme
Pseud.sp. Lipase

Fig. 6

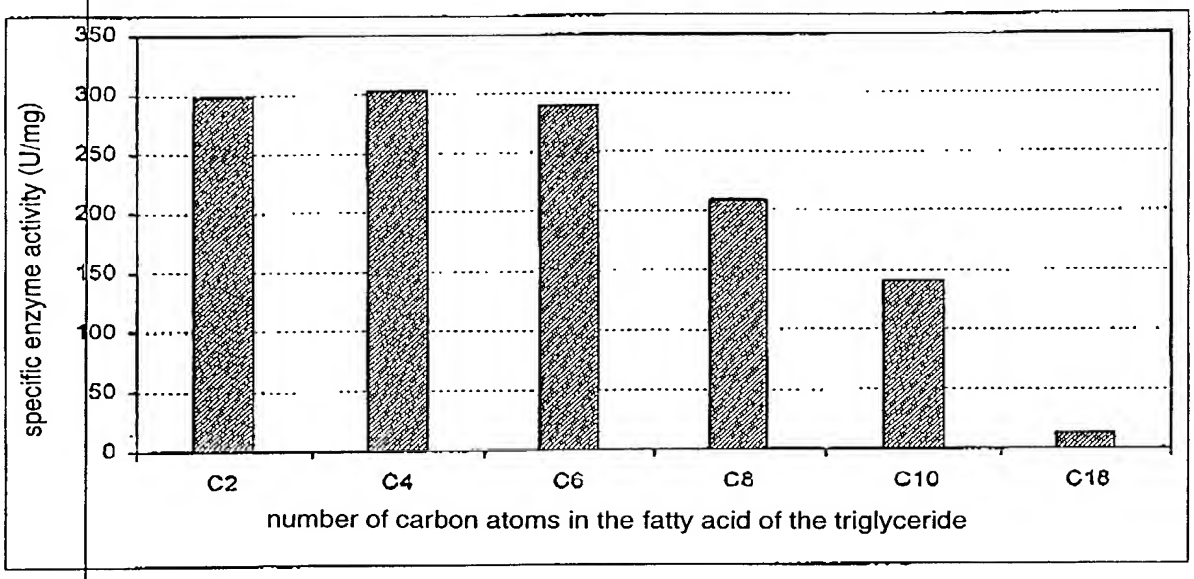
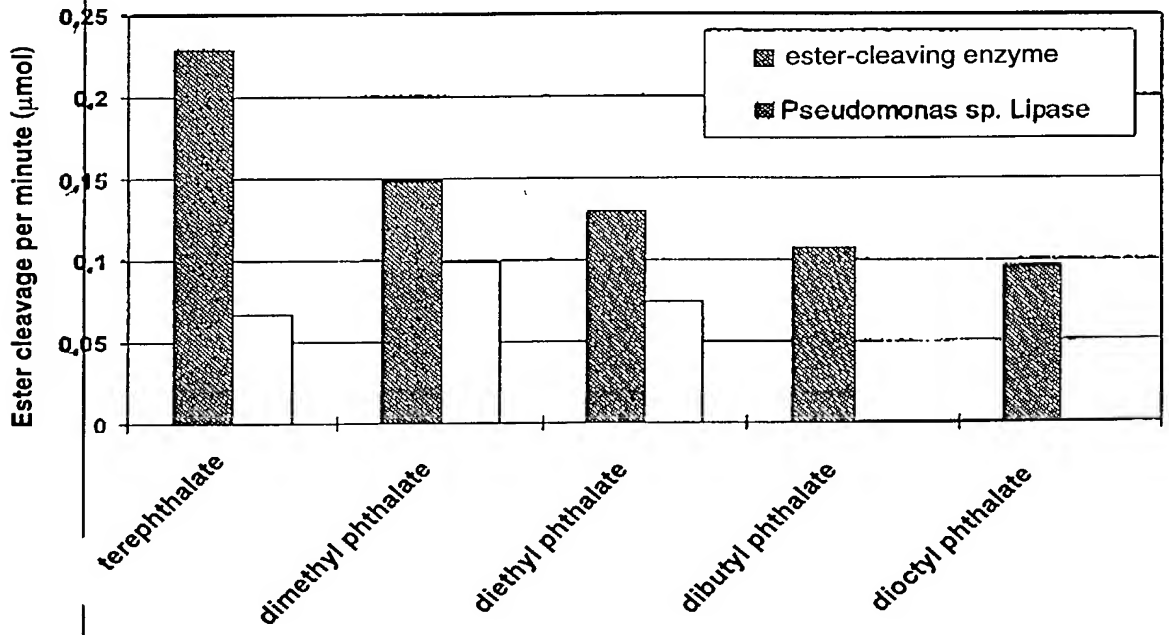

specific enzyme activity (U/mg)
350
300
250
200
150
100
50
0
C2
C4
C6
C8
C10
C18
number of carbon atoms in the fatty acid of the triglyceride

Fig. 7


Ester cleavage per minute (µmol)
0,25
0,2
0,15
0,1
0,05
0
ester-cleaving enzyme
Pseudomonas sp. Lipase
terephthalate
dimethyl phthalate
diethyl phthalate
dibutyl phthalate
dioctyl phthalate

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (1)

(Under 37 CFR § 1.63; includes reference to PCT International Applications)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention ENTITLED:

ESTER-GROUP-CLEAVING ENZYME FROM THERMOMONOSPORA

the specification of which:

- ☐ is attached hereto
- ☐ was filed on _____ as:
- ☐ United States Application Serial No. 10/089,392
- ☒ as a National Phase or Continuation or Continuation-in-Part or Divisional of PCT Application No. PCT/EP00/07115, filed July 25, 2000 and designating the U.S., and published as _____ on
- ☐ with amendments through _____ (if applicable, give details).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT International application(s) designating at least one country other than the United State of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT International applications designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign/PCT Application(s) [list additional applications on separate page]:

Country (or PCT)	Application Number:	Filed (Day/Month/Year)	Priority Claimed: Yes	No
Germany	DE19947286.6	30/09/1999	☒	☐
			☐	☐

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States application listed below:

_____
(Application Number) (Filing Date)

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Express Mail No. EV073651651US

00058480

[redacted]

Prior U.S. (or U.S.-designating PCT) Application(s) [list additional applications on separate page]:

U.S. Serial No.: Filed (Day/Month/Year) PCT Application No. Status (patented, pending, abandoned)

1

I hereby appoint Ronald R. Santucci, Registration No. 28,988, and Frommer Lawrence & Haug LLP, or their duly appointed associate, my attorneys, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to file continuation and divisional applications thereof, to receive the Patent, and to transact all business in the Patent and Trademark Office and in the Courts in connection therewith, and to insert the Serial Number of the application in the space provided above, and specify that all communications about the application are to be directed to the following correspondence address:

Ronald R. Santucci, Esq.
c/o FROMMER LAWRENCE & HAUG LLP
745 Fifth Avenue
New York, NY 10151

Direct all telephone calls to: (212) 588-0800
to the attention of: Ronald R. Santucci

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1.00 INVENTOR(S):

Signature: Wolf Dieter Deckwer Date: 06/07/02
Full name of sole or first inventor: Wolf-Dieter Deckwer
Residence: Mascheroder Weg 1, 38124 Braunschweig, Germany
Citizenship: German
DEX

2.00 Signature: Rolf-Joach. Mu Date: 19.06.02

Full name of 2nd joint inventor (if any): Rolf-Joachim Mueller
Residence: Mascheroder Weg 1, 38124 Braunschweig, Germany
Citizenship: German
DEX

3.00 Signature: Ilona Kleeberg Date: 20.06.02

Full name of 3rd joint inventor (if any): Ilona Kleeberg
Residence: Mascheroder Weg 1, 38124 Braunschweig, Germany
Citizenship: German
DEX

4.00 Signature: 14 June 2002 Date: 14 June 2002

Full name of 4th joint inventor (if any): Joop van den Heuvel
Residence: Mascheroder Weg 1, 38124 Braunschweig, Germany
Citizenship: Dutch
DEX

00058480

[redacted]